Biomarkers for Toxicology Studies
September 20-21, 2006

The NTP sponsored a workshop to identify additional biomarkers of lung disease, heart disease, and altered carbohydrate and lipid metabolism (e.g., metabolic syndrome) that could be assessed routinely in NTP subchronic toxicology studies to better characterize endpoints of environmentally-induced disease or biological process related to human disease etiology. The workshop organizers selected these areas of focus after surveying major human diseases and disorders with respect to mortality, incidence, economic impact, and relationship to environmental factors. Additional information about the meeting, including background materials and presentations are available on the NTP website at http://ntp.niehs.nih.gov/go/20940.

Workshop Recommendations:
Lung:
The lung breakout group felt the three most useful approaches were: (1) bronchoalveolar lavage analysis, (2) enhanced histopathology (especially the addition of trichrome stain, Periodic Acid Schiff stain, Ki67 for cell proliferation), and (3) gene expression analysis. Because of concerns on analyzing and interpreting large scale gene expression data the group recommended the NTP explore the use of gene expression analysis not as a routine measure, but on a more limited basis. Additionally, the group believed that imaging techniques held great promise but are still in the late stages of refinement.

Heart:
The heart breakout group recommended the routine inclusion of three biomarkers to in NTP subchronic studies: (1) cardiac troponin, (2) a2-macroglobulin in the rat, and (3) B-type natriuretic protein in conjunction with ultrasound. While troponin is sensitive and specific for myocardial injury, assay selection is paramount for obtaining valid results. Although not specific for cardiac injury, a2-macroglobulin (analogous to human CRP) was recommended because the group felt it important to have an indication of systemic inflammation. BNP assays based on serum samples are available for humans. In rodents the assay requires RNA extraction from the heart so the group suggested that the NTP develop a serum assay for rodents. Ultrasound and imaging studies were also recommended for consideration.

Lipid/Carbohydrate Metabolism:
The three highest priority biomarkers identified by the lipid/carbohydrate metabolism breakout group were (1) serum cholesterol/triglycerides, (2) insulin, and (3) glutathione. Though not indicative or predictive of a disease process per se and though the information from rodents does not bridge to human applications, triglyceride concentrations would reasonably indicate whether the animal is eating or not. And, cholesterol in rodents (which is primarily HDL) does appear to track with changes in total cholesterol in humans (though lipoprotein fraction changes are not similar between humans and rodents). Insulin would be a better indicator of insulin resistance than glucose. And, as an alternative, the breakout group suggested that NTP consider measuring red blood cell hemoglobin A1c or serum fructosamine (these assays are less

sensitive to feeding status). While not specific for disorders of lipid or carbohydrate metabolism, reduced glutathione analysis was recommended as a marker of whole body oxidative stress; other attendees voiced concerns suggesting the analysis and data interpretation would be problematic. The group also indicated that body composition analysis, using dual-energy x-ray absorptiometry to evaluate lean mass, fat mass and bone density or microCT to distinguish visceral and subcutaneous fat, was recommended either for routine use on a subset of animals or in special studies. Enhanced histopathology (really, refined classification terminology – e.g., discriminating between microvesicular and macrovesicular fatty change in the liver) was also recommended on a routine basis.